

## A REPORT ON THE GROWTH OF EXCISED TOMATO ROOTS

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*With one plate*

It was found by Robbins and Schmidt (5, 6, 7) that a solution of mineral salts, cane sugar and thiamine (or the vitamin thiazole, 4-methyl-5 $\beta$ -hydroxyethyl thiazole) appeared adequate for the unlimited growth of excised tomato roots. The first report (5, 7) covered 12 successive passages extending over a period of one year in a solution of mineral salts, cane sugar and synthetic thiamine and seven successive passages in a period of seven months in the same solution with thiazole substituted for thiamine. The second report (10) was made at the end of 29 passages in the thiamine solutions and 23 in those containing thiazole. We have now maintained this strain of roots through 125 passages for a period of ten years and ten months since the roots were severed from the original seedlings on September 29, 1935. The clones have been grown for 112 successive passages in a solution of mineral salts, cane sugar and thiamine and for 105 successive passages in a solution of mineral salts, cane sugar and thiazole.

These experiments are of interest because of the extended period of culture. Some additional observations have been made on this strain of roots, which are also recorded here.

## CONDITIONS OF CULTURE

The excised roots were grown individually in 50 ml. of solution in 125 ml. Erlenmeyer flasks of Pyrex glass. The mineral salts were of C. P. grade; the cane sugar, Pfanstiehl's C. P. sucrose; the thiamine, Merck's synthetic. The 4-methyl-5 $\beta$ -hydroxyethyl thiazole was obtained through the courtesy of Merck & Co. It was free from vitamin pyrimidine as determined by tests with *Phycomyces blakesleeanus*. This is an important consideration, as we have had samples of thiazole contaminated with pyrimidine and others have reported to us similar difficulties. All glassware was cleaned with sulfuric-chromic acid cleaning mixture and thoroughly rinsed with tap and distilled water. A comparison of the growth of excised tomato roots in glassware cleaned with sulfuric-chromic acid cleaning mixture and in glassware cleaned with concentrated hydrochloric acid showed no differences. We concluded that the residual effects of chromium (3) were not important in these experiments.

For the last several years the roots have been cultivated in a modified Pfeffer's solution plus one per cent cane sugar and 10 m $\mu$  moles of thiamine or of thiazole per flask. The modified Pfeffer's solution was prepared

by diluting stock solutions of the various salts. Our procedure was as follows: The stock solutions consisted of

I.	Ca(NO <sub>3</sub> ) <sub>2</sub> ·4 H <sub>2</sub> O .....	83.25 g.
	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> .....	0.565 g.
	Distilled water .....	500 ml.
II.	KCl .....	20.8 g.
	KNO <sub>3</sub> .....	41.6 g.
	KH <sub>2</sub> PO <sub>4</sub> .....	41.6 g.
	MgSO <sub>4</sub> ·7H <sub>2</sub> O .....	41.6 g.
	Distilled water .....	500 ml.
III.	H <sub>3</sub> BO <sub>3</sub> .....	2.86 g.
	MnSO <sub>4</sub> ·4H <sub>2</sub> O .....	2.04 g.
	or	
	MnCl <sub>2</sub> ·4H <sub>2</sub> O .....	1.81 g.
	ZnSO <sub>4</sub> ·7H <sub>2</sub> O .....	0.22 g.
	CuSO <sub>4</sub> ·5H <sub>2</sub> O .....	0.08 g.
	H <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O .....	0.09 g.
	Distilled water .....	1000 ml.

Two ml. of stock solution I, 1 ml. of II and 0.1 ml. of III were added to 1000 ml. of distilled water.

The final solution contained per liter 333 mg. Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 41.6 mg. KCl, 83.2 mg. KNO<sub>3</sub>, 83.2 mg. KH<sub>2</sub>PO<sub>4</sub>, 83.2 mg. MgSO<sub>4</sub>·7H<sub>2</sub>O, 2.26 mg. Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, 0.286 mg. H<sub>3</sub>BO<sub>3</sub>, 0.181 mg. MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.022 mg. ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.008 mg. CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.009 mg. H<sub>2</sub>MoO<sub>4</sub>·H<sub>2</sub>O. The approximate amounts of the supplemental mineral elements in parts per million in this solution were 0.32 Fe, 0.05 B, 0.05 Mn, 0.005 Zn, 0.002 Cu and 0.005 Mo.

The iron precipitated in stock solution I but by shaking satisfactory aliquots could be obtained. The other stock solutions remained clear and without precipitate.

The amount of sugar was found to be quite important. One per cent sugar was much superior to the two per cent used in the early passages (Pl. I, upper). The growth of replicate cultures was more uniform; the individual roots were more normal in appearance and showed less browning.

The modified Pfeffer's solution containing cane sugar can be autoclaved at 12 pounds pressure for 20 minutes with no deleterious effects as far as the growth of the tomato roots is concerned and with minor inversion of the cane sugar.

Transfers to fresh culture media were made at approximately monthly intervals. A portion, about 0.5 cm. square of a safety razor blade spot-welded on an iron wire held in a Rosenberger and Greenman needle holder was used to cut the roots into pieces. The pieces of root were one or two cm. long and included the primary root tip or one or more branch tips. The pieces were transferred to fresh culture media by a platinum wire bent into an L at the tip. This was found to be more convenient than a straight wire or one with a loop on the end.

For some years the roots were incubated in faint diffuse light at 25°C. Since the incubator was not equipped to run at temperatures below room temperature there were periods during the summer months when the incubator temperature exceeded 25°, rising to as much as 30° for part of some days. Some difficulty was experienced at times in maintaining the thiazole cultures. This we suspect may have been caused by the higher temperatures of the summer months. Our cultures are now being maintained at 20° in the dark. They are exposed to light occasionally for short periods when observations are made.

Growth has been measured by dry weights usually at the end of two months' growth. The roots were washed with distilled water, placed in weighed aluminum pans, dried at 100°C., cooled and weighed. The results for passages 84 to 96 inclusive are given in TABLE 1. The average

TABLE 1.

Growth of excised tomato roots in modified Pfeffer's solution, 1 per cent cane sugar and 10  $\mu$  moles of thiamine or thiazole through 13 successive passages.

Date	Passage	Supplement	No. Roots Weighed	Dry Wt. per root mg.	Range Dry Wt. mg.
10/7/42 to	84	Thiamine	20	6.5	4.9- 9.8
12/10/42		Thiazole	5	7.8	5.1-10.0
11/9/42 to	85	Thiamine	18	9.5	6.2-15.2
1/8/43		Thiazole	23	5.7	1.5-10.4
.....	86	No weights taken			
1/12/43 to	87	Thiamine	49	10.5	5.0-16.1
3/24/43		Thiazole	31	6.0	1.0-11.7
2/11/43 to	88	Thiamine	3	8.6	6.0-13.4
4/16/43		Thiazole	12	5.6	2.3- 8.7
3/12/43 to	89	Thiamine	6	9.8	8.1-12.3
5/13/43		Thiazole	23	7.7	4.6-10.7
4/13/43 to	90	Thiamine	19	9.4	6.8-12.0
6/12/43		Thiazole	25	9.9	0.9-15.1
5/11/43 to	91	Thiamine	2	9.8	.....
7/19/43		Thiazole	23	12.0	.....
6/10/43 to	92	Thiamine	24	7.8	.....
8/10/43		Thiazole	24	12.9	.....
7/6/43 to	93	Thiamine	3	8.8	.....
9/9/43		Thiazole	22	13.8	.....
8/4/43 to	94	Thiamine	14	13.8	.....
10/11/43		Thiazole	25	13.2	.....
9/6/43 to	95	Thiamine	21	9.2	.....
11/9/43		Thiazole	12	16.2	.....
10/6/43 to	96	Thiamine	23	13.4	.....
12/10/43		Thiazole	24	8.8	.....

dry weights of the roots varied somewhat from passage to passage. In the 12 passages given the lowest average dry weight in the thiamine solutions was 6.5 mg. and the highest 13.3 mg.; for the thiazole solutions these figures were 5.6 mg. and 16.2 mg.

## RELATION TO VITAMINS

Robbins and Schmidt demonstrated by the use of *Phycomyces blakesleeanus* that this strain of tomato roots synthesizes the pyrimidine portion of thiamine or a substitute therefor. This accounts for the ability of these roots to grow indefinitely in a medium supplemented with the vitamin thiazole only.

Reid and Robbins (2) found the excised roots grown in a thiamine solution produced ascorbic acid.

I have found the roots to synthesize biotin and pyridoxine in solutions supplemented with thiamine or thiazole. This was demonstrated as follows:

Roots which had grown 57 days in the thiamine solution in passage 73 were immersed in a medium containing per liter 1.5 g.  $\text{KH}_2\text{PO}_4$ , 0.5 g.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 50 g. dextrose, 2.0 g. asparagine and 1.5 per cent purified agar. Zero, one or three roots were added per tube containing 8 ml. of the agar medium. The average dry weight per root was 6.8 mg. After sterilization one set of tubes was inoculated with *Ceratostomella ulmi* and another with *Ceratostomella ips* #438; *C. ulmi* has a complete deficiency for pyridoxine and *C. ips* #438 for biotin (4). The only source of vitamins in the medium was the tomato roots. The growth of *C. ulmi* demonstrated the presence of pyridoxine or a substitute for it and that of *C. ips* #438 the presence of biotin (PL. I, lower).

A similar experiment performed with roots grown in a thiazole solution showed the synthesis of biotin and pyridoxine in that medium also.

The demonstration that these tomato roots synthesize pyridoxine in a solution of mineral salts, cane sugar and thiamine or thiazole explains their ability to grow in a solution containing no pyridoxine. They require pyridoxine and in its absence from the medium synthesize enough for some growth though not enough for maximum growth.

Our determinations of pyridoxine in excised tomato roots were not quantitative. However, it is reasonable to suppose that the marked improvement in growth noted (8, 9) when pyridoxine is added to the thiamine medium is because the amount of pyridoxine synthesized by the roots is inadequate. In the thiamine solution growth is limited by the amount of pyridoxine synthesized and the addition of the latter vitamin to the medium permits more growth to occur.

*Pyridoxine, Pyridoxal and Pyridoxamine.* Snell (11) found that pyridoxal and pyridoxamine were as effective for some organisms as pyridoxine and in some instances were considerably more so. For the strain of tomato roots discussed in this paper pyridoxal and pyridoxine were equally effective under the conditions of our experiments. Pyridoxamine may be somewhat less active since as much dry weight was found with 10  $\mu$  moles of the first two compounds as with 50  $\mu$  moles of pyridoxamine. The difference, however, is not great and is not of the same order of magnitude as found by Snell (11) for some organisms, for example, *Lactobacillus casei* and

*Streptococcus lactis* R. Our excised tomato roots are apparently able to convert these compounds into the functional one whatever that may be.

In the experiments summarized in TABLE 2 the pyridoxal and pyridoxamine were filtered sterile and added to the balance of the solution after it

TABLE 2.

Dry weights of excised tomato roots grown two months in 50 ml. of modified Pfeffer's solution containing one per cent cane sugar, 10 mμ moles of thiamine and the amounts of pyridoxine, pyridoxamine or pyridoxal given in the table.

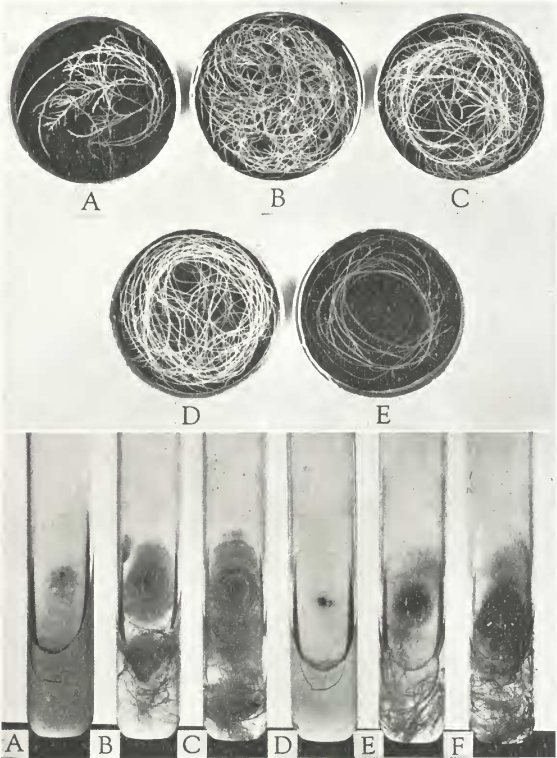
Supplement in mμ moles	Pyridoxine			Pyridoxamine			Pyridoxal		
	No. Roots	Av. dry wt. mg.	Range mg.	No. Roots	Av. dry wt. mg.	Range mg.	No. Roots	Av. dry wt. mg.	Range mg.
100	9	27.2	22.2-29.3	9	27.9	24.9-30.9	10	28.5	26.1-29.8
50	10	24.8	18.4-28.5	9	24.3	19.7-27.3	10	26.0	22.6-29.4
10	9	25.5	21.3-29.7	10	17.1	15.1-19.9	7	24.2	22.6-28.3
1	10	14.5	11.2-20.4	7	12.7	10.7-14.6	9	14.7	12.0-17.1
0.1	8	13.0	11.1-14.5	10	13.5	10.6-16.1	9	15.2	8.4-18.3
0.0	9	14.3	12.4-17.7	9	14.3	12.4-17.7	9	14.3	12.4-17.7

had been autoclaved. The inoculum came from roots grown 24 days in a thiamine solution in passage 107. The roots were incubated in faint diffuse light.

We might expect considerable destruction of these compounds in diffuse light in a period of two months on the basis of the findings of Cunningham and Snell (1). Although it would be desirable to repeat our experiments with roots grown in the dark, we doubt whether our conclusions on the relative effectiveness of the three compounds would be changed. We have been unable to detect a difference in the dry weights of excised tomato roots grown two months side by side in the dark and in the light in solutions containing thiamine or thiamine and pyridoxine.

During this extended period of culture (more than 100 passages) we have observed no evidence that the roots have changed genetically. They appear to be growing now about as they did in 1939 or earlier. Neither has there been any sign of the production of shoots, although we would expect shoot production to be within their genetic potentialities.

We have produced individual roots with dry weights of 200 mg. or more. These were grown 106 days at room temperature in diffuse light in liter flasks containing 100 ml. of the modified Pfeffer's solution and one per cent cane sugar plus 30 mμ moles of thiamine and 166 mμ moles of pyridoxine. In a period of two months in 50 ml. of solution supplemented with 10 mμ moles of thiamine and 50 mμ moles of pyridoxine the maximum



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